ZOOLOGICAL SCIENCE **10**: 385–392 (1993)

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REVIEW

Proliferation of Anterior Pituitary Cells in Relation to Aging and Longevity in the Rat

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ABSTRACT—Some of the hormones secreted from the anterior pituitary are related to the potency of survival during the aging process. The proliferation of thyrotrophs affects the lifespan of the rat through the activity of the thyroid gland. Adrenocorticotropic hormone promotes the deterioration of brain function in aged rats. Average prolactin levels and the incidence of prolactinoma increase in aged rats. A longitudinal study showed that the increase in prolactin levels represents the precancerous state for a prolactinoma. Culture experiments indicate that the proliferation of acidophils is regulated by hypothalamic growth hormone-releasing factor and other hormones, which are possibly responsible for the age-related change in acidophil populations.

ANTERIOR PITUITARY CELLS AND THE RATE OF AGING IN THE RAT

Theories on the biological mechanisms of aging include the alteration of the humoral regulatory system in which the hypothalamo-pituitary axis has central importance. In this chapter I will deal only with the role of the anterior pituitary in the aging of structures and functions related to the survival of individuals. The anterior pituitary is deeply concerned in age-related changes in the reproductive system. With regard to these aspects, other review articles can be consulted [2, 20, 28, 77].

In his series of research on surgically hypophysectomized rats, Everitt [19] has shown that several cirteria for aging (hardening of tail tendon collagen, increase in protein excretion in urine, renal and cardiac hypertrophy, etc.) are prevented by hypophysectomy. Despite the retarded rate of aging in these criteria, hypophysectomy shortens the duration of life. Later work showed, however, that replacement therapy with cortisone acetate prolongs the lifespan of hypophysectomized rats to even longer than the lifespan of intact rats, thereby suggesting that adrenocorticotropic hormone (ACTH) is an essential life-maintaining factor in survival [21]. From these results he concluded that the pituitary secretes "aging hormone(s)" which accelerate the rate of aging processes.

Chronic food restriction has been the most effective means for prolonging the lifespan of rats [30, 46]. The onset of various kinds of age-associated disease is also delayed by food restriction. Some studies have shown that food restriction inhibits the secretion of pituitary hormones [13, 48], suggesting that the anti-aging effect of food restriction may partly be ascribed to the reduced secretion of "aging hormone(s)".

One candidate for such an aging factor is the pituitary-thyorid axis. A very early study showed that mice fed desiccated thyroid throughout their liver had shorter lifespans than controls [67]. Exposure to low temperature (9°C), which was expected to induce the secretion of thyroxine (T_4), shortened the lifespan compared to control rats maintained at 28°C [35]. The periodic acid—Schiff (PAS) stainability of the anterior pituitary decreased in tryptophan-deficient rats, which also showed retardation of aging. The concentrations of T_4, 3,3′,5-triiodothyronine (T_3), and thyrotropic

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Received December 25, 1992

hormone were lower in these tryptophan-deficient animals than in control rats [57].

More direct and precise experiments were carried out using the neonatally T_4-treated hypothyroid rats of both sexes. Thyrotrophs in the anterior pituitary of neonatal rats proliferate actively and increase rapidly in number [55]. When rats were injected intraperitoneally with 1–2 μg T_4 per g body weight 5 times during the first 10 days of life, the division of thyrotrophs was suppressed by the exogenous T_4. There was almost no increase in the number of thyrotrophs in rats at 10 days of age. The capacity to increase the number of thyrotrophs was restored after discontinuation of T_4 injection; the pituitaries in adult rats treated neonatally with T_4 contained almost the same density of thyrotrophs as the controls [55]. The levels of T_4 in the treated rats were lower than in control rats, as reported in previous studies [3, 5], throughout almost the whole lifespan, indicating that the function of the thyroid gland was impaired by the inhibition of thyrotroph proliferation during neonatal T_4-treatment. The levels of T_3 were also lower than controls in young rats. These artifitial hypothyroid rats of both sexes lived longer than control rats [56]. On the other hand hyperthyroid rats, produced by continuous administration of T_4 in the drinking water, had shorter lifespans than controls [58]. The life-shortening effect of T_4 was observed even when the rats were treated with T_4 during only the first or second half of life. T_4 treatment was not effective, however, when treatments were confined to the senescent period (more than 26 months old). These results indicate that T_4 does not directly induce the senescent diseases that are the causes of death, but accelerates the aging rate during the young and middle-aged periods [58].

The aging factors suggested by the experiments of Everitt seem not to include ACTH, because he supplied glucocorticoid to hypophysectomized rats that showed aging retardation. However, it has been reported that the ACTH-adrenal cortex axis accelerates some aspects of the aging process. Wexler [91] has reported the premature aging of the cardiovascular system and many other tissues in hyperadrenocorticoid rats produced by repeated breeding. Recent evidence indicates that some aging phenomena in the hippocampus and other brain regions are modulated by glucocorticoids [23]. Sapolsky [71] emphasized that the hippocampus is a primary CNS target for glucocorticoids which induce neuronal loss in the aged hippocampus. Minimal damage in the hippocampus was observed in adrenalectomized rats after microinjection of toxins (kainic acid or 3-acetylpyridine). Enhanced damage was induced in corticosterone-treated rats. Adrenalectomy appears to protect the brain against age-related reductions in neuronal density and age-related increases in glial density [44]. However, part of the effect of adrenalectomy seems to be due to prolonged neural stimulation resulting from elevated ACTH, rather than to a reduction in steroids *per se*, since the morphologic correlates of brain aging were reduced and maze learning was improved by treatment with a non-steroidogenic ACTH analog.

AGE-ASSOCIATED DEVELOPMENT OF HYPERPROLACTINEMIA AND PROLACTINOMA

The concentration of prolactin (PRL) in the blood of female rats increases with age [78, 84]. The incidence of spontaneous prolactinoma also increases in the pituitary of aged rats, but there is a wide variation among strains [93]. High tumor incidence in one strain is generally associated with age-related hyperprolactinemia, not only in the individuals which have pituitary hyperplasia, but also in the rats with histologically normal pituitaries [89]. Although aged male rats of many strains also have high PRL levels, the magnitude differs depending on the strain [7, 65, 70, 90]. In mice, C57BL/6J females over 14 months of age have a high tumor incidence [22], but PRL levels are not elevated in male mice at ages up to 28 months [17].

Even in strains in which aged female rats have very high average PRL levels, the individual variations are very large with some rats showing low PRL values at advanced ages [89]. Therefore it is impossible to determine any chronological pattern for the development of hyperprolactinemia or prolactinoma in individual rats from the cross-sectional data. A longitudinal study on PRL levels in female Wistar rats by serial sampling of blood from the same individuals was useful for such purpose [59]. A prolactinoma inevitably develops within 3 months in rats with blood PRL levels higher than 70 ng/ml. On the other hand, rats with no tumors at the time of death showed very low PRL levels throughout life (Fig. 1). These facts indicate that moderate hyperprolactinemia is the precancerous state prior to the development of prolactinoma. The duration of tumor growth is 2–3 months (Fig. 1). The highest generation of new tumors is observed at around 27 months of age. Some rats with a large tumor survive for more than 10 months, indicating that prolactinoma is not always a direct cause of death for rats [59]. This finding may have some relation to a report that increased immunoreactive PRL molecules show low biological activity when assayed for proliferation-inducing potency on Nb_2 lymphoma cells [12]. Moreover, we can expect anti-aging effects of PRL since the administration of PRL prevents an age-related decrease in dopamine receptors in the rat striatum [68]. PRL-treated rats recovered from motor dysfunctions.

As with many other aging criteria, the age-associated increase in PRL levels is suppressed in rats by food restriction [81]. Synthesis of PRL messenger RNA is also reduced in energy-restricted C3H/SHN F_1-hybrid mice [41]. These suppressions might be due to direct inhibition of the production or secretion of pituitary hormones by underfeeding, since the level of growth hormone (GH), which decreases with age, is remarkably reduced by food restriction [62]. Measurements of PRL levels in rats refed after 24 months of food restriction by an intermittent food supply, however, showed that low PRL levels are maintained for more than 9 weeks, indicating that the prevention of PRL increase in food-restricted rats is not due to a direct effect of underfeeding, but represents a retardation of aging in the hypothalamo-pituitary axis (Fig. 2).

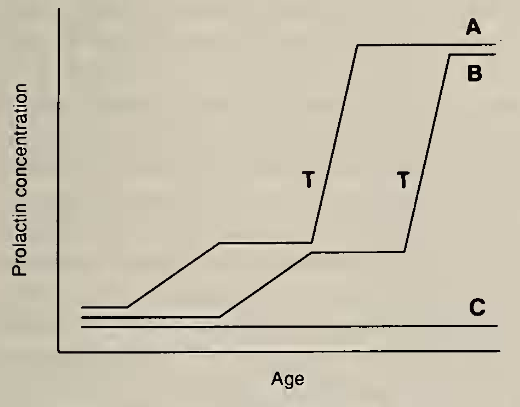

FIG. 1. Schematic representation of the longitudinal changes in the PRL levels of female Wistar rats with large tumors (A), small tumors (B), and no tumors (C) at the time of death. The time of tumor development was accompanied by a sudden rise in PRL level (T). Prolactinomas always developed in hyperprolactinemic rats within 3 months. When the period between tumorigenesis and examination of the pituitary was less than 2 months, the pituitary had a small tumor (less than 1 mm), indicating that the duration of tumor growth is about 2 months.

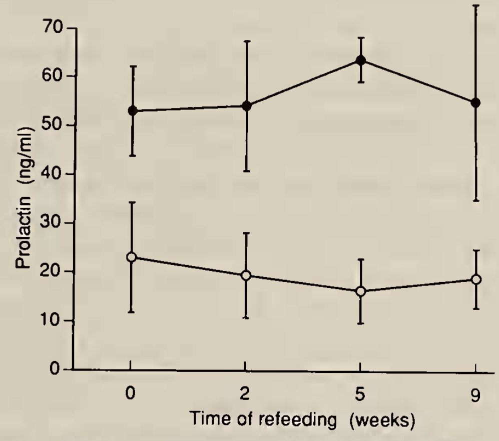

FIG. 2. PRL levels in food-restricted and refed male Fischer 344 rats. Experimental rats (open circles) were fed every other day from 1 to 24 months of age, with a total food intake approximately 60% that of controls (closed circles). Radioimmunoassay for PRL was performed on serially sampled blood from the same individuals. Protection against hyperprolactinemia continued for more than 9 weeks of *ad libitum* refeeding.

The close association of hyperprolactinemia with prolactinoma suggests the possibility that the number of mammotrophs increases in the pituitary of hyperprolactinemic rats. The volume fraction occupied by the mammotrophs in the pituitary increases with age [89]. The ratio of mammotroph number to total secretory cells also increases in the pituitary of hyperprolactinemic rats [15], although no increase was observed in aged individuals with low PRL levels [59]. Moreover, many previous studies have shown that the synthesis and secretion rate of PRL in each mammotroph do not increase, or rather decrease in the pituitary of aged rats [86]. These results indicate that changes in the regulation of cell number, besides the regulation of hormone secretion [6, 18, 27, 33, 37, 40, 53, 54, 82], are important in the mechanism for the development of hyperprolactinemia.

REGULATION OF ACIDOPHIL PROLIFERATION IN AGING AND TUMORIGENESIS

Mammotrophs in the pituitary of adult animals can increase through cell proliferation, or by conversion from somatotrophs [38]. The possibility of transdifferentiation of mammotrophs and somatotrophs is suggested by the presence of cells which secrete both PRL and GH (mammosomatotrophs) [29, 75]. Estrogen induces the production of PRL in somatotrophs, and increases the number of mammosomatotrophs in primary pituitary cell culture obtained from adult male rats [10] and in a clonal pituitary cell line, GH_3 [9]. Insulin and IGF-1 stimulate the secretion of PRL, and inhibit the secretion of GH, in GH_3 cells [49, 61]. On the other hand, cortisol antagonizes insulin in the secretion of PRL by GH_3 cells [61]. Cortisol also increases the number of both somatotrophs and mammosomatotrophs in cultured bovine pituitary cells by transdifferentiation [39]. More detailed informations about mammosomatotrophs are available in a recent review [86].

The increase in mammotrophs by cell proliferation, however, is most likely in age-related hyperprolactinemia, since tissue hyperplasia is a general feature of the precancerous state. The proliferative activity of mammotrophs is stimulated by estrogen, which also enhances the secretion of PRL [32, 47, 78]. Both the mitotic index and the number of cells in S-phase increase after *in vivo* estradiol treatment of rats [34, 60, 87]. Prolonged administration of natural or synthetic estrogen induces prolactinomas in rats and mice [11, 92]. The sustained plasma estradiol levels associated with persistent vaginal cornification in middle-aged rodents are suspected by some authors to be a factor responsible for both the elevated PRL levels and the high incidence of prolactinoma in females [23]. In constant estrous Long-Evans rats older than 25 months, moderately high estrogen levels are accompanied by high PRL concentration in the blood [45]. However, plasma estrogen levels are rather lower in constant estrous C57BL/6J mice than in the estrous and diestrous stages of cyclic young in which estrogen levels are very high in the proestrous stage [22]. Moreover, only a small portion of rats older than 25 months are in constant estrus [1, 45], while many rats show an increase in PRL levels and prolactinoma development between 25 and 30 months [59]. In some strains of rats, for example, Wistar and Fischer 344 rats, age-related hyperprolactinemia and prolactinoma are observed even in males [7]. These facts suggest the presence of other factors involved in the increase in mammotroph number.

Hypothalamic dopamine is a well-known inhibitor of PRL secretion. Sulpiride, a dopamine antagonist, activates both DNA synthesis and the mitosis of mammotrophs *in vivo*, and bromocriptine, a stable dopamine agonist, suppresses them [34, 87]. The content of dopamine in the hypothalamus decreases in aged rodents [16, 80]. Old female rats with spontaneous prolactinomas, as well as young females with prolactinomas induced by prolonged estrogen treatment, show damaged tuberoinfundibular dopaminergic neurons [72–74]. These reports suggest that dopamine plays an important role in the regulation of mammotroph proliferation. However, the sensitivity of mammotroph DNA synthesis to amantadine, a compound that causes an increase in dopamine synthesis, seems to be lower than that ot PRL secretion [42]. Dopamine concentration in the hypophyseal portal blood has been reported to decrease with age in both male [26] and female [64] rats, but a more recent study has shown that the rate of dopamine secretion is markedly elevated in aged rats [31]. Dopamine content in the anterior pituitary is also higher in aged rats than in young rats [63].

Among secretory cells in the anterior pituitary of adult rats, mammotrophs are the only cells to proliferate actively in culture with ordinary basal medium containing no hypothalamic factors [4]. Recent studies on cultured anterior pituitary cells have revealed the direct influence of hormones, especially hypothalamic peptides, on the proliferation of these cells. Billestrup *et al.* [8] reported that DNA synthesis in cultured somatotrophs is stimulated by the addition of growth-hormone releasing factor (GRF), and suppressed by somatostatin. Frawley and Hoeffler [24] studied the effects of various hypothalamic hormones on the number of mammotrophs and somatotrophs in 5-day-old rats by a reverse-plaque assay. They reported that the relative number of mammotrophs to somatotrophs increases in cultures containing GRF or luteinizing hormone-releasing hormone (LHRH). The proliferation of both cell types is stimulated by corticotropin-releasing factor (CRF), but thyrotropin-releasing hormone (TRH) stimulates the proliferation of other cell types. Treatment of pituitary reaggregate cell culture from immature (14-day-old) rats with LHRH or neuropeptide Y increased ^{3}H-thymidine incorporation into mammotrophs and corticotrophs, whereas these peptides decreased the number of labeled somatotrophs [88]. The actions of these peptides were suggested to be mediated by specific paracrine growth factors released from gonadotrophs.

The effects of various hypothalamic hormones on the proliferation of pituitary cells from adult rats were examined in culture using 8-month-old female Wistar rats [79]. In a basal medium consisting of Eagle's MEM and 10% fetal bovine serum, mammotrophs proliferated actively, and no increase in the number of somatotrophs was observed. The proliferative situation was completely reversed by the addition of GRF (Fig. 3). This result suggests that the relative number of acidophils is regulated by hypothalamic GRF through inverse responses of mammotrophs and somatotrophs to GRF. GH and PRL belong to the same gene family, and both secretory cells differentiate from the same precursor cells [38]. The mechanism by which the expression of one gene changes the characteristics of cell proliferation is worth investigating. Another possibility is that GRF suppresses the proliferation of mammotrophs indirectly through an unknown inhibitor secreted by somatotrophs or other cell types under the stimulation of GRF. Failure to detect GRF binding sites on the mammotroph surface suggests this possibility [51]. If this assumption is correct, the postulated inhibitor should be specific to mammotrophs since the proliferation of somatotrophs is not suppressed. In contrast with the effect of dopamine, GRF does not inhibit the secretion of PRL [14, 76, 83].

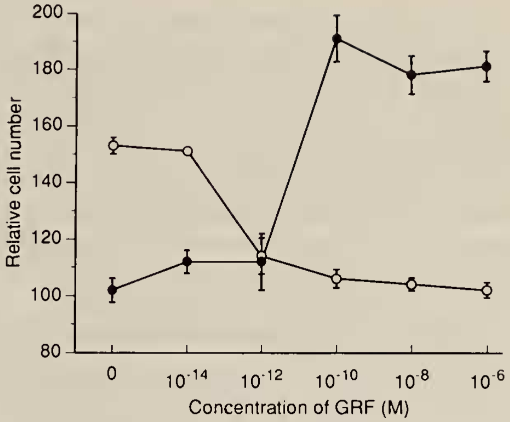

Fig. 3. Changes in the numbers of mammotrophs (open circles) and somatotrophs (closed circles) from adult rats during a 6 days in culture with varying concentration of GRF. Relative cell number represents the percentage of cells at the start of culture period.

The content of GRF in the median eminence decreases markedly with aging in the rat [52]. This is not conclusive evidence that GRF secretion into the protal blood is reduced, since a decreased content of a substance in the median eminence could occur with either increased or decreased secretion. However, the fact that the secretion of GH [62] decreases with aging, while total volume of GH cells does not change [86], suggests a decrease in GRF secretion in aged rats. It is possible that a decrease in GRF secretion plays a role in the change in the relative number of acidophils in aged rats.

The addition of LHRH into the culture accelerates the rate of mammotroph proliferation (submitted for publication). It has been reported that the content [69] and secretion rate [85] of LHRH increase gradually in middle-aged and old rats. High luteinizing hormone (LH) levels in the blood of aged mice also suggest an increase in LHRH secretion in these animals [25]. On the other hand, no increase is observed in the blood LH levels of aged female rats [45]. However, a decrease in the responsiveness to LHRH may prevent the increase in LH release in aged rats, since the density of LHRH receptor is reduced in the pituitary cells of aged rats [50]. From these data it can be assumed that LHRH also contributes to the age-related increase in the number of mammotrophs.

Although the proliferation of mammotrophs in adult rats is activated by LHRH, the stimulation of PRL secretion by physiological doses of LHRH seems to be restricted to very young animals [66]. In older rats, the PRL-secreting response to high, nonphysiological doses of LHRH has been reported, and this phenomenon is suggested to be mediated by angiotensin II produced by paracrine secretion [36, 43]. But angiotensin II had no distinct effect on the proliferation of mammotrophs in culture experiments.

The suppressive effect of bromocriptine on mammotroph proliferation has been confirmed *in vitro* (unpublished data). TRH stimulates the secretion of PRL [27, 33], but showed no effect on the proliferation of mammotrophs.

The effects of hypothalamic and ovarian hormones on the proliferation of acidophils, as shown by *in vitro* studies, are summarized in Fig. 4. Age-related changes in the production and secretion of these hormones probably cause the changes in the acidophil populations of the anterior pituitary in aged rats, the increase in mammotrophs and the decrease in somatotrophs.

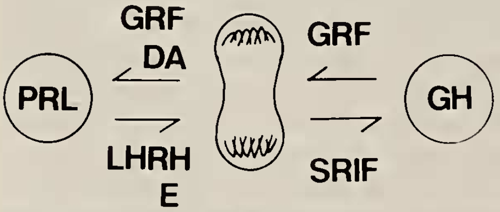

Fig. 4. Regulation of the proliferation of mammotrophs (PRL) and somatotrophs (GH) by GRF, LHRH, dopamine (DA), somatostatin (SRIF), and estrogen (E).

ACKNOWLEDGMENTS

The author is grateful to Dr. T. Shinkai, Department of Cell Biology, Tokyo Metropolitan Institute of Gerontology, for his cooperation in the preparation of this review. Hormones and antibodies for radioimmunoassay and immunostaining were kindly supplied by the National Institute of Diabetes and Digestive and Kidney Diseases, USA, and Gunma University, Japan.

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